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CHARACTERIZATION OF REAEROSOLIZATION FROM IMPINGERS IN AN EFFORT TO IMPROVE AIRBORNE VIRUS SAMPLING

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14. ABSTRACT

An AGI-30 impinger containing particles (MS2 bacteriophage or 30-nm polystyrene latex (PSL)) of known concentration was operated with sterile air. Reaerosolized particles as a function of sampling flow rate and impinge collection liquid concentration were characterized using a Scanning Mobility Particle Sizer. Reaerosolization from the impinge was also compared to that from a BioSampler. Results show that the reaerosolization increases as flow rate increases. While the increased particle concentration in the impinge collection liquid leads to an increase in the reaerosolization of PSL particles, it does not necessarily lead to an increase in the reaerosolization of virus particles. Reaerosolization of virus particles begins to decrease as the particle concentration in the impinge collection liquid rises above 10^6 PFU/mL. This phenomenon likely results from the aggregation of viral particles at high concentrations. Compared with micron-sized particles, nanosized virus particles are easier to aerosolize due to reduced inertia. Reaerosolization from the BioSampler is demonstrated to be significantly less than that from the impinge. Reaerosolization from impingement sampling methods is a mode of loss in airborne virus sampling, although it is not as significant a limitation as the; primary particle size of the aerosol. Utilizing a BioSampler coupled with shorter sampling periods to prevent high accumulative concentrations can minimize the impact of reaerosolization. This study confirms reaerosolization of virus particles to be a mode of loss in impingement sampling and identified methods to minimize the loss.

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CHARACTERIZATION OF REAEROSOLIZATION FROM IMPINGERS IN AN EFFORT TO IMPROVE AIRBORNE VIRUS SAMPLING

By

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ABSTRACT

AIMS: To assess the impact of reaerosolization from liquid impingement methods on airborne virus sampling

Methods and Results: An AGI-30 impinger containing particles (MS2 bacteriophage or 30-nm polystyrene latex (PSL)) of known concentration was operated with sterile air. Reaerosolized particles as a function of sampling flow rate and particle concentration in the impinger collection liquid were characterized using a Scanning Mobility Particle Sizer. Reaerosolization from the impinger was also compared to that from a BioSampler. Results show that reaerosolization increases as flow rate increases. While the increased particle concentration in the impinger collection liquid leads to an increase in the reaerosolization of PSL particles, it does not necessarily lead to an increase in the reaerosolization of virus particles. Reaerosolization of virus particles begins to decrease as the particle concentration in the impinger collection liquid rises above 10^6 PFU/mL. This phenomenon likely results from aggregation of viral particles at high concentrations. Compared with micron-sized particles, nanosized virus particles are easier to aerosolize due to reduced inertia. Reaerosolization from the BioSampler is demonstrated to be significantly less than that from the impinger.

Conclusions: Reaerosolization from impingement sampling methods is a mode of loss in airborne virus sampling, although it is not as significant a limitation as the primary particle size of the aerosol. Utilizing a BioSampler coupled with short sampling periods to prevent high accumulative concentrations can minimize the impact of reaerosolization.

Significance and Impact of Study: This study confirms reaerosolization of virus particles to be a mode of loss in impingement sampling and identifies methods to minimize the loss.

Keywords: reaerosolization, virus, sampling, impinger, aggregation

INTRODUCTION

The perceived threat of bioterrorism and airborne virus outbreaks, including historical epidemics of influenza and more recent occurrences of SARS and various strains of influenza, have led to heightened concern about the prevalence and potential effects of airborne viruses (Rengasamy et al., 2004). Although there is increased attention to these potentially deadly microorganisms (Ratnesar–Shumate et al. 2008; Lee et al. 2008), current bioaerosol sampling methods are unable to effectively sample airborne viruses. Sampling efficiency of current bioaerosol sampling methods is less than 10% for the most challenging sizes of 30–100 nm (Hogan et al. 2005), a significant concern with respect to the common size of a virus (20–300 nm) (Madigan et al. 2003). If sampling methodologies do not provide accurate results, the discrepancy between measured and actual virus concentrations could potentially lead to disastrous decision errors because the infectivity of viruses is measured as minimum infectious dose to 50% of a population (MID_{50}). For example, the MID_{50} for smallpox is 10–100 through inhalation and for Ebola is < 10 (Public Health Agency of Canada). Therefore, understanding the limitations that lead to poor airborne virus sampling is crucial.

A typical sampling system for airborne viruses utilizes an impactor, liquid impinger, or filter (Tseng and Li 2005) to physically collect the viruses to subject them to viability analysis. Liquid impingement is a commonly adopted method, as it lends itself nicely to viral enumeration techniques (Terzieva et al. 1996; Hogan et al. 2005). Liquid impingement methods carry the air stream around a tight turning radius in an attempt to separate particles with higher inertia from the air stream. However, recent experiments with liquid impingers, including an AGI-30 impinger, a BioSampler, and a frit bubbler, demonstrated that less than 10% of particles in the 30–100 nm range were collected (Hogan et al. 2005). Collection efficiency is highly dependent

on particle size, and particles in the 30–100 nm range had neither sufficient inertia nor sufficient diffusion to be collected well in the impinger.

Along with particle size, reaerosolization has been indicated to be a potential limitation for bioaerosol sampling (Willeke et al. 1995). Reaerosolization occurs when collected particles in the collection liquid are re-entrained into the air stream, which leads to decreased collection efficiency. Even though impingement is the established method for airborne virus sampling (Lin et al. 1997; Dart and Thornburg 2008), reaerosolization is a common concern in impinger operation. The turbulence associated with impinger operation provides enough energy for particles to become re-entrained into rising bubbles (Willeke et al. 1995; Grinshpun et al. 1997).

Theoretical models for aerosol transfer from bubbles to the surrounding liquid are available and are largely based on Fuchs' work presented in *The Mechanics of Aerosols* (1964). The theory sums the contributions of mass transfer from multiple removal mechanisms. For small particles, diffusion is the main removal mechanism. For larger particles, inertia and sedimentation become increasingly important (Ghiaasiaan and Yao 1997). The coefficients of deposition due to diffusion (α_d) and inertia (α_i) are presented in Eqs. 1 and 3, and definitions for all variables are provided in the Nomenclature list.

$$\alpha_i = \frac{9U_B\tau}{2R_B^2} \tag{1}$$

$$\tau = \frac{2\rho_p R_p^2}{9\mu} \tag{2}$$

$$\alpha_d = 1.8\left(\frac{D}{R_B^3 U_B}\right)^{1/2} \tag{3}$$

$$D = \frac{kT_G C_C}{6\pi\mu R_p} \tag{4}$$

Following these theoretical expressions, it is apparent that inertial removal of aerosols from bubbles into a surrounding liquid clearly plays a larger role as particle size (R_p) increases

and as bubble velocity (U_B) increases. Similarly, diffusive removal of particles from a bubble into a surrounding liquid increases with increasing diffusivity as R_p decreases, as the bubble radius (R_B) decreases, and as U_B decreases, which allows more time for diffusion to occur. The overall effect of mass transfer of aerosols from a bubble to a liquid is very similar to the passage of aerosols through filters (Fuchs 1964; Pich and Schutz 1991). The deposition of aerosols within a filter as a function of particle size will have a clear minimum because large aerosols collect well by inertial transfer and small particles collect well by diffusive transfer.

Both mechanisms must be re-examined to study the reverse effect, such that particles in the liquid are transferred from the liquid into the bubble, as is the case during reaerosolization. The case for diffusion will be a similar situation. Mass transfer from the liquid into the bubble will increase as diffusivity of the particle increases and more time is allowed for diffusion to occur. Of course, the total diffusive transfer will also depend on the concentration of particles in the liquid. The case for inertial mass transfer will be nearly opposite. In this scenario, the amount of inertia required to re-entrain a particle into a bubble will increase as particle size increases. Therefore, mass transfer of aerosols into the bubble due to inertia will increase as particle size decreases. Combining these two, it presents that the mass transfer of particles into bubbles from liquid to enable reaerosolization will be significantly larger for smaller particles because diffusion will be increased and the inertial requirement to re-entrain the particles will be smaller, *i.e.*, the resultant deposition is therefore dictated by α_i.

A study on reaerosolization of *Bacillus cereus* bacterial spores from liquid impingers (Grinshpun et al., 1997) observed no bacterial reaerosolization even with intense bubbling at 10 Lpm. At 12.5 Lpm, several super-micrometer particles were detected downstream of the impinger outlet, and the collection efficiency decreased from 100% at 10 Lpm to 80–90% at 12.5

Lpm. A separate experiment observed significantly more reaerosolization of the bacterial spore as flow rate increased from 5 to 12.5 Lpm. They concluded that the decrease in impinger collection efficiency of the *B. cereus* bacterial spore at 12.5 Lpm was due to reaerosolization. Theoretical models presented by Fuchs (1964) and Ghiaasiaan and Yao (1997) predicted this observation, by theorizing that increased bubble rise velocity introduces more inertia into the system, thereby enhancing the removal of aerosols from a liquid.

Lin et al. (1997) found that reaerosolization of polystyrene latex (PSL) particles in the size range 0.51 μm to 1.60 μm increased with sampling time as the concentration of collected particles in the impinger collection liquid increased. After approximately 30 minutes of sampling, reaerosolization started to steadily increase as collection liquid was removed by aerosolization from the impinger. After 60 minutes of sampling, the amount of reaerosolization significantly affected the collection efficiency of the impingers. They noted that reaerosolization was much more dependent on the concentration in the collection liquid than on the incoming airborne concentration. This trend can be explained with the theoretical discussions because the concentration gradient between the liquid and bubble will increase as the concentration in the collection liquid increases, thereby increasing diffusive mass transfer into the bubble and enhancing reaerosolization. The collection of 0.51-μm particles was much more impacted by reaerosolization than was the collection of 1.02-μm or 1.60-μm particles; nearly 10% of all 0.51-μm particles were reaerosolized after 60 minutes of sampling.

The experimental observation that reaerosolization increases as particle size decreases warrants investigation on a viral aerosol, which is a smaller particle than those examined in prior studies. Reaerosolization from impinger collection liquid can be expected to antagonize airborne virus collection efficiency, although the extent of this effect is not known. Therefore, the

objective of the study was to investigate reaerosolization of virus-sized particles from the impinger to assess the impact of this mode of loss on airborne virus sampling. Logical hypotheses for virus particles were that reaerosolization would increase as both flow rate and accumulative concentration in impinger collection liquid increased, and that reaerosolization for virus-sized particles would be greater than that for bacterium-sized particles. While operational flow rate is often established by sampling protocol, the accumulative impinger concentration increases with sampling time. The results from these experiments will provide recommended sampling time limits based on accumulative impinger concentration that can be established to minimize the effects of reaerosolization. Furthermore, virus reaerosolization from an AGI-30 impinger was compared to that from a BioSampler.

MATERIALS AND METHODS

Experiments to determine reaerosolization as a function of flow rate and impinger concentration followed experimental methods similar to those used in previous work to characterize bacterial reaerosolization (Willeke et al. 1998; Lin et al. 2000; Hogan et al. 2005). Figure 1 shows a schematic of the experimental system. Particles of known concentration were placed in the impinger collection liquid, and the AGI-30 impinger (Ace Glass, Inc., Vineland, NJ, USA) was operated with sterile cylinder air (relative humidity < 20%) at room temperature ($23 \pm 2\ ^{o}C$). The flow rate through the system was controlled by a rotameter, which was calibrated with a Gilibrator (Scientific Instrument Services, Inc.). The flow exiting the impinger carried any aerosolized droplets and reaerosolized particles downstream. A slip stream directed 0.6 Lpm of the impinger exhaust flow through a diffusion dryer to remove excess moisture and finally into the Scanning Mobility Particle Sizer (SMPS, Model 3936, Shoreview, MN, USA), which measured the reaerosolized particles . A baseline test using pure deionized (DI) water (0

PFU/mL) in the impinger collection liquid prior to every experiment was used to confirm that the experimental setup was operating properly. The nebulizer in the baseline test hypothetically produced pure water droplets, from which the diffusion dryer removed any moisture, and the SMPS registered negligible aerosol particles. Any aerosols detected in the baseline were due to low levels of dissolved solids present in the DI water, and the total reaerosolized concentrations were accordingly adjusted. Three sets of experiments were conducted to analyze reaerosolization of virus-sized particles. Details of each set are discussed in the following subsections.

Reaerosolization with PSL

The first experiment utilized PSL particles (Duke Scientific, Palo Alto, CA, 3030A, nominal 30-nm particles, density 1.05 g/cm^3) in the impinger liquid to provide a preliminary analysis of reaerosolization of virus-sized particles. DI water was used to dilute the PSL particles to the desired concentrations (0.1, 1.0, 10, 100 ppm by volume), and the impinger was operated at the recommended operational flow rate of 12.5 Lpm (Ace Glass Inc. 2008). Five tests at each scenario were measured by the SMPS over a 12-minute period. Airborne virus particles are difficult to distinguish from residual solute particles caused by the liquid medium, and the liquid medium typically dictates the particle size distribution of the airborne virus (Hogan et al. 2005). Viruses also have inherent microbial uncertainties, including loss of viability and microbial interactions, which can influence their behavior. The use of PSL particles in DI water eliminated these uncertainties and provided a simpler and more straightforward test. Therefore, this test was conducted to obtain very explicit results regarding reaerosolization of virus-sized particles.

Reaerosolization with MS2

Although PSL particles provided explicit information regarding the reaerosolization of virus-sized particles, the test needed to be conducted with a virus to see if the results from PSL translated to an actual virus. Thus, the reaerosolization experiment was conducted much more thoroughly with MS2 virus in the impinger liquid to obtain results specific to the virus. MS2 is a bacteriophage that is often used as a surrogate for airborne virus testing and is an appropriate choice for use as a surrogate human pathogenic virus (Aranha–Creado and Brandwein 1999). The nominal size of the MS2 bacteriophage is 27.5 nm (Golmohammadi et al. 1993); thus, it serves as a suitable challenge for airborne virus sampling studies because typical collection efficiencies in the impinger at this particle size are less than 10% (Hogan et al. 2005). MS2 is classified as a hydrophilic virus because of the absence of a lipid envelope surrounding the nucleocapsid (Vidaver et al. 1973; Madigan et al. 2003; Tseng and Li 2005). In this research, MS2 was prepared using freeze-dried viruses from ATCC (*Escherichia coli* bacteriophage ATCC® 15597-B1™).

To determine the effect of flow rate and particle concentration in the impinger collection liquid on reaerosolization, experiments were run at three flow rates (9, 12.5, and 15 Lpm) for each concentration (0, 10, 10^2, 10^4, 10^6, and 10^8 PFU/mL). Preliminary infectivity tests were run during some of the initial reaerosolization tests. The time span of the reaerosolization tests (< 15 minutes) is a common time frame for sampling and is within recommended limits to minimize liquid evaporation and microbial damage (Terzeiva et al, 1996; Lin et al, 1997).

One concern for the reaerosolization tests with MS2 was the decision to use DI water instead of phosphate buffered saline (PBS) as the impinger liquid. Although PBS (1.8 g KH_2PO_4, 15.2 g K_2HPO_4, and 85 g NaCl in 1L of DI water) is often used in bioaerosol sampling to maintain bioaerosol viability, the salt aerosols formed when the liquid is aerosolized can mask

the magnitude of reaerosolized virus particles. Similarly, it is difficult to distinguish aerosolized viruses from residual solute particles formed from aerosolized liquid medium that is used to maintain virus viability. The media typically dictate the airborne virus particle size distribution (Hogan et al. 2005). The experiments attempted to minimize the effects of background media such as PBS and virus stock medium, but the issue was inherent to the task at hand regardless of these efforts. Twenty μL of PBS was inherently included in 20 mL of virus suspension in the process of preparing the impinger liquid.

Reaerosolization from BioSampler

The last experiment was a test comparing reaerosolization from the AGI-30 impinger to that from the BioSampler (SKC, Inc., Eighty-Four, PA). Previous research indicated that the BioSampler produced significantly less reaerosolization of particles in the bacterial size range due to the swirling motion during collection (Willeke et al. 1998; Lin et al. 2000). This experiment attempted to confirm that this was also the case for particles in the viral size range. Reaerosolization of 30-nm PSL particles from the BioSampler was compared to that from the AGI-30 impinger using conditions identical to the first set of experiments.

RESULTS

Reaerosolization with PSL

Figure 2 displays the reaerosolized concentration of PSL particles. The results have been adjusted for concentrations measured during the baseline experiment (DI water). The measured concentrations for the subsequent tests are therefore displayed as adjusted reaerosolized concentration.

As shown, the amount of PSL particle reaerosolization increased consistently as the particle concentration in the collection liquid increased. An analysis of variance (ANOVA) test verifies that each increase at the higher concentrations (from 1 to 10 ppm and from 10 to 100 ppm) was deemed significant ($p < 0.05$), which agrees with prior studies (Lin et al. 1997) utilizing larger PSL particles and the theoretical prediction.

Reaerosolization with MS2

The dependence of the reaerosolization of MS2 bacteriophage particles on flow rate and the particle concentration in the collection liquid was evaluated. The average mode particle size at each concentration and flow rate is presented in Table 1. The average mode for the baseline test is the smallest due to the droplets being composed of pure DI water. The average mode at 10^8 PFU/mL is the next smallest, due to loss of aerosolizable viruses to larger aggregated particles. Mean reaerosolized MS2 concentration as a function of impinger concentration for each of the three flow rates tested (9, 12.5, 15 Lpm) are displayed in Figure 3. As shown, increasing the flow rate significantly increased the number of virus particles reaerosolized. Increased concentration, however, did not necessarily lead to an increase in reaerosolization for virus particles. Rather, the count of reaerosolized virus particles increased as concentration increased until it reached a concentration of approximately 10^6 PFU/mL, above which the reaerosolized count began to decrease. The trends at the lower flow rates followed the same pattern, but the overall level of reaerosolization was minimal in comparison to the higher flow rates. This agrees with Fuchs' work, the application of which predicted that increasing flow rate will increase reaerosolization.

Statistical analysis was run on these tests using the ANOVA test. The difference between reaerosolized concentrations is generally significant ($p<0.05$) between the baseline (10^0

PFU/mL) and the middle concentrations (10, 10^2, 10^4, 10^6 PFU/mL). The three middle concentrations (10^2, 10^4, 10^6 PFU/mL) are generally not significantly different ($p>0.05$). The reaerosolized amount is generally significant ($p<0.05$) between the middle concentrations (10^2, 10^4, 10^6 PFU/mL) and the highest concentration (10^8 PFU/mL).

In general, reaerosolization increased significantly as flow rate increased. The increasing trend agreed with the hypothesis that extra inertia will reaerosolize more particles. The unexpected result was the decrease in reaerosolization at concentrations higher than 10^6 PFU/mL. For all flow rates, the highest levels of concentration resulted in a decrease in reaerosolization. Although this was not expected, the observation may be explained by changes in aggregation and surface tension due to changes in concentration of proteins and salt in the DI water and virus stock. This will be discussed more thoroughly in the Discussion section.

Reaerosolization from BioSampler

Reaerosolization of 30-nm PSL particles from the BioSampler at various concentrations in the collection liquid was compared to that from the AGI-30 impinger. The results are shown in Figure 4 as adjusted mean reaerosolized concentration from the three tests. Percent change from the impinger to the BioSampler at each concentration is also displayed. The level of reaerosolization of PSL particles from the BioSampler is clearly shown to have been significantly lower than that from the impinger. Reaerosolization from the BioSampler was generally two orders of magnitude lower than that from the impinger under identical conditions.

DISCUSSION

Reaerosolization with PSL

Analysis of the reaerosolization of 30-nm PSL particles provided very direct documentation of virus-sized particle reaerosolization. Reaerosolization of virus particles is complicated by proteins, salts, and complex microbial interactions, all of which make it difficult to distinguish naked reaerosolized virus particles. In contrast, the results from the PSL particles in DI water are explicit: reaerosolization of virus-sized particles occurs and can be a mode of loss. This agrees with previous research on reaerosolization completed for particles in the bacterial size range (Grinshpun et al. 1997; Lin et al. 1997; Willeke et al. 1998; Dart and Thornburg, 2008).

Another benefit of conducting the experiment with PSL particles is that comparison with previous work can be made. The level of reaerosolization for virus-sized particles was compared to previous work done on reaerosolization for particles in the bacterial size range under similar conditions. A simple analysis was carried out by "normalizing" these results for comparison. Table 2 displays the comparison. Note that the simple analysis normalizes the peak mode aerosol concentration to the concentration in the impinger collection liquid, as the total reaerosolized concentrations for the previous literature were not reported. As shown, a 30-nm particle is much more likely to be reaerosolized than a 0.5- or 1.0-μm particle at similar number concentrations. This corroborates the expectation based on Fuchs' work (1964) that smaller particles are more likely to be reaerosolized because the energy requirements to re-entrain them are lower and their diffusivity is higher.

While it is clear that there is more reaerosolization of the 30-nm particles for similar number concentrations in the impinger ($10^8 – 10^9$ #/mL), the analysis was also conducted for similar mass concentrations, assuming unit densities for the bacterial species. The 100 ppm (10^{12} particles/mL) scenario in the present research had a mass concentration similar to that in Willeke

et al. (1998) (10^{-4} g/mL vs. 5×10^{-5} g/mL). Based on mass concentrations, the "normalized" reaerosolization was closer to that of larger particles (6×10^{7} particles/mL for Willeke et al. 1998). Furthermore, it was less than that at lower concentration (1.2×10^{7} particles/mL at 100 ppm vs. 8.9×10^{6} particles/mL at 0.1 ppm). Aggregation of the PSL particles at very high concentrations might be one possible reason.

Table 3 displays the percentages of particles reaerosolized. Although reaerosolization occurs and results in downstream total concentrations that seem to be high, less than 1.0% of the particles contained in the impinger were reaerosolized over the sampling period at each concentration tested. Furthermore, the loss, expressed as percentage of particles reaerosolized, became less significant as the concentration increased. This number cannot be compared to previous research for bacterium-sized particles because the total concentrations for those scenarios were not provided in the literature.

Reaerosolization is expected to increase with sampling time (Lin et al. 1997) due to water vaporization. However, the present work indicates that reaerosolization may not be significant (<1%) over short sampling periods (less than 15 minutes in the present work). Therefore, if the initial physical collection limitations due to particle size can be overcome, significant improvements in airborne virus sampling can be achieved.

Further analysis of the PSL reaerosolization data was attempted to provide more insight into the aerosolization from the impinger. Although the impinger is not designed to be an aerosol generator, in practice it naturally acts as one due to the formation of liquid aerosols during operation. Equation 5 was used to determine whether the extent of reaerosolization is volumetrically proportional to the concentration in the impinger collection liquid (Hinds 1999).

(5)

The equation states that aerosols with particle volume concentration V_p can be generated based on the droplet volume concentration V_d produced by the impinger and the volume fraction of solid material in the impinger liquid, F_v. The volumetric analysis based on triplicate particle measurements from the SMPS is also displayed in Table 3. As shown, the consistency between experiments was strong. At the lowest concentration (0.10 ppm), the average droplet volume was significantly larger than at the higher concentrations (1.0, 10, 100 ppm). This was likely due to the stronger effect of the residual dissolved solids (*e.g.*, stabilizers in the PSL stock suspension from the manufacturer). The higher concentrations showed strong consistency, with approximately 2×10^{14} nm^3/cm^3 of droplet aerosol generated from the impinger at 12.5 Lpm. This confirms the hypothesis that the impinger was essentially operating as an aerosol generator and was able to consistently generate a specific droplet volume.

Reaerosolization with MS2

The results from the reaerosolization of MS2 convey the extent to which this phenomenon is a concern for bioaerosol samplers, especially in the submicrometer size range. The increase in reaerosolization as a function of flow rate is not surprising; it is in agreement with previous work by Grinshpun et al. (1997) with bacteria as well as theoretical models based on Fuchs' (1964) work on aerosol transfer between bubbles and surrounding liquid. The theory indicates that increased bubble rise velocity introduces more inertia into the system, thereby causing reaerosolization. In contrast, the trends observed as concentration increased were unexpected. The initial increase in reaerosolization with increased concentration seems logical, but the subsequent decrease in reaerosolization at the highest concentration tested was unexpected based on the literature review. Most bioaerosols in natural systems do not generally exist in very high concentrations, and the time limitations on impingers due to the evaporative nature of the

collection liquid prevent long-term sampling (Lin et al. 1997). Thus, a particle concentration in the collection liquid as high as 10^8 PFU/mL is unlikely in most sampling scenarios. Regardless, the phenomenon is interesting, and science compels further explanation.

One possible explanation is that the viruses were present in an aggregated state at the highest concentrations, thereby increasing the effective particle size and making reaerosolization more difficult. Figure 5 displays the scanning electron microscopic (SEM) images of particles from virus suspension of 10^8 PFU/mL of DI water. Particles of around 1 μm are present (Fig 5a), and a closer look (Fig 5b) clearly shows the presence of aggregated particles. Investigation into the subject of viral aggregation provides information about the frequent state of aggregation based on viral and external factors. In aqueous scenarios, it is common for viruses to aggregate at high concentrations (Floyd and Sharp 1977; Grant 1994). External factors, such as salt concentration and *p*H, can also affect the level of aggregation (Floyd and Sharp 1977; Floyd and Sharp 1978). Generally, aggregation is dependent on the virus concentration, ionic strength, and *p*H of the liquid.

Virus concentration in an aqueous solution contributes to the level of aggregation. Aggregation occurred when Floyd and Sharp (1977) prepared reovirus and poliovirus suspensions with 1:10 dilutions in DI water from stock solutions of 7×10^{11} and 2×10^{12} particles/mL, respectively, as physically counted by electron microscopy. Any further dilution (1:100 or 1:1000) resulted in dispersed viruses. The concentration of viruses in natural water is typically very low, but this is not always the case in laboratory scenarios, including the concentrations used in the present work. At 10^8 PFU/mL the particle concentration in the collection liquid is sufficient to cause aggregation of MS2 virus as seen in Figure 5.

Literature suggests that an increase in salt concentration can significantly decrease aggregation. Floyd and Sharp (1977) found that the level of ionic strength required for their version of PBS to prevent aggregation was approximately 10 mM for poliovirus. They concluded that aggregation could occur even with appreciable salts present. As only a very small amount of PBS was added (0.01 mL 10X PBS) in the present study, there probably was not sufficient salt concentration to prevent viral aggregation.

Hydrophobic interactions between proteins from neighboring viruses also lead to the formation of aggregates. Although MS2 is labeled a hydrophilic virus because of the absence of a lipid envelope surrounding the nucleocapsid, it can still experience these hydrophobic interactions in aqueous solutions (Thomas et al. 1998; Hogan et al. 2004). In fact, Shields and Farrah (2002) found that MS2 experienced strong hydrophobic interactions during adsorption to solids, even though the absence of the lipid envelope indicates general hydrophilic behavior. The hydrophobic tendencies of the MS2 might be especially manifested at high concentrations.

The effect of *p*H on aggregation is based on the isoelectric point of the virus. Generally, *p*H values below the isoelectric point result in aggregation, while *p*H values above generally do not (Floyd and Sharp 1977; van Voorthuizen et al. 2001). The isoelectric point of MS2 bacteriophage is 3.9 (van Voorthuizen et al. 2001), and the *p*H of the impinger liquid was close to neutral. Thus, the *p*H in this scenario probably did not contribute to aggregation of viral particles as much as the other factors.

Reaerosolization is expected to increase with sampling time. This is due to an increase in accumulated concentration in the impinger collection liquid as operation time increases and a decrease in the amount of collection liquid because of evaporation and aerosolization (Lin et al. 1997). The high concentration of MS2, the limited salt, and the tendency for MS2 to initiate

hydrophobic interactions with one another result in viral aggregation in the impinger liquid that explains the phenomenon of decreased reaerosolization at high ($>10^6$ PFU/mL) particle concentration in the collection liquid. This places yet another constraint to the use of the AGI-30 impinger for sampling airborne viruses. However, the likelihood of reaching very high impinger concentrations ($>10^5$ PFU/mL) is low before much of the 20 mL of initial collection liquid evaporates. Thus, reaerosolization of MS2 may not be as significant a factor contributing to poor airborne virus sampling as the effect due to virus particle size.

Reaerosolization from BioSampler

The significant decrease in the reaerosolization of 30-nm PSL particles from a BioSampler in comparison to that from an AGI-30 impinger corroborated past work done on the subject for bacterium-size particles. Table 2 displays results from the current work as well as the previous work using bacterium-sized particles. Willeke et al. (1998) used 0.5- and 1.0-µm PSL particles in 20 mL of DI water in both samplers to compare reaerosolization from the two methods of liquid impingement. The BioSampler performed significantly better, with a peak mode concentration of reaerosolized particles lower than 4 particles/cm^3. In comparison, the AGI-30 had a peak mode concentration nearly 60 particles/cm^3. Similarly, Lin et al. (2000) showed that the peak mode concentration of *Pseudomonas fluorescens* vegetative cells and *Bacillus subtilis* spores from the BioSampler was about 20% of that from the AGI-30. The improved performance in reduced reaerosolization by the BioSampler is attributed to the swirling motion in which the air travels. The entrance of the air in the AGI-30 is perpendicular to the base of the collection vessel, which allows for much more bubbling and aerosolization of the collection liquid (Willeke et al. 1998).

Although Hogan et al. (2005) noted that even the BioSampler cannot exceed 10% physical collection in the 20–300 nm size range, the use of a BioSampler minimizes reaerosolization of virus-sized particles. Thus, the low physical collection efficiency for virus particles seen in the BioSampler is likely due to insufficient initial physical collection rather than reaerosolization. This is likely due to the ability of nanosized particles to tolerate the centrifugal collection motion and escape collection. Based on this information, if viral primary particle size can be increased, the BioSampler should significantly improve airborne virus sampling by increasing physical collection and minimizing reaerosolization issues.

CONCLUSIONS

Reaerosolization occurs for virus-sized particles and is more of a concern for virus-sized particles than for bacterium-sized particles, as demonstrated explicitly by the use of 30-nm PSL particles. Reaerosolization increases as flow rate increases, due to the additional energy introduced to the system. While increased concentration leads to increased reaerosolization for PSL particles, increased concentration does not necessarily lead to an increase in reaerosolization for virus particles. Rather, reaerosolization of virus particles increases as concentration increases until it reaches a concentration of approximately 10^6 PFU/mL, above which concentration reaerosolization begins to decrease. One reason for the observed phenomenon is aggregation of viral particles resulting from high virus concentration, low salt concentration and hydrophobic interaction. Nevertheless, such high concentrations are not common due to typical airborne virus concentrations and liquid impingement sampling limitations. Reaerosolization of virus-sized particles does not appear to be a significant mode of loss during 15 minutes of sampling for most typical sampling scenarios, and it can be minimized by preventing high impinger concentrations and using the BioSampler. It is also deduced from the study that the low physical collection

efficiency in the BioSampler is due to insufficient initial physical collection rather than reaerosolization.

Recommendations to improve airborne virus sampling based on the present work include the use of the BioSampler, potentially in conjunction with a size amplification apparatus to increase primary particle size of the airborne virus. Based on previous work of liquid evaporation from impinger operation, it is recommended that sampling last no more than 30 minutes.

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NOMENCLATURE

Symbol	Title	Units
α_i	Coefficient of deposition due to inertia	1/m
α_d	Coefficient of deposition due to diffusion	1/m
U_B	Bubble velocity	m/s
τ	Relaxation time	s
R_B	Bubble radius	m
R_P	Particle radius	m
ρ_p	Particle density	kg/m^3
μ	Gas viscosity	Pa-s
K	Boltzmann constant	$1.3x10^{-23}$ J/K
T_G	Temperature of gas	K
C_c	Cunningham's correction factor	Dimensionless
D	Aerosol diffusivity	m^2/s

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Table 1 Comparison of mean mode sizes for MS2 reaerosolized from AGI-30 Impinger

	Mean Mode Size (nm)		
Virus Concentration in the Collection Liquid (PFU/mL)	9 Lpm	12.5 Lpm	15 Lpm
0	16	12	11
10^2	61	79	51
10^4	59	81	48
10^6	61	80	31
10^8	33	45	34

Table 2 Comparison of reaerosolization for bacterium-sized particles to that for virus-sized particles from AGI-30 Impinger and BioSampler

	Current work (0.1 ppm)	Current work (100 ppm)	Willeke et al. 1998	Lin et al. 2000	Lin et al. 2000
Particle Type	PSL	PSL	PSL	*P. fluorescens*	*B. subtilis*
Particle Size (μm)	0.03	0.03	0.5, 1.0	0.8	1.0
Number Conc. in AGI-30 (#/mL)	1×10^{9}	1×10^{12}	1×10^{8}	1×10^{8}	1×10^{8}
Mass Conc. in ImpingerAGI-30 (g/mL)	1×10^{-7}	1×10^{-4}	5×10^{-5}	2×10^{-5}	5×10^{-5}
Peak Mode Conc. from AGI-30 (#//cm^3)	8,900	120,000	60	5	55
Normalized Peak Mode Conc. from AGI-30	8.9×10^{-6}	1.2×10^{-7}	6.0×10^{-7}	5.0×10^{-8}	5.5×10^{-7}
Peak Mode Conc. from BioSampler (#//cm^3)	N/A	1,600	4	1	10
Peak Mode Conc. Ratio of BioSampler to AGI-30	N/A	0.013	0.067	0.200	0.182

Table 3 Median percentage of PSL particles reaerosolized and estimated droplet volume concentration generated from impinger

Particle Concentration in the Collection Liquid (ppm)	% Particles Reaerosolized	Droplet Volume Concentration at 12.5 Lpm (nm^3/cm^3)
0.10	0.84%	$1.1\times10^{15} \pm 5.4\times10^{14}$
1.0	0.10%	$2.2\times10^{14} \pm 1.1\times10^{14}$
10	0.03%	$1.8\times10^{14} \pm 1.5\times10^{13}$
100	0.01%	$2.0\times10^{14} \pm 6.4\times10^{13}$

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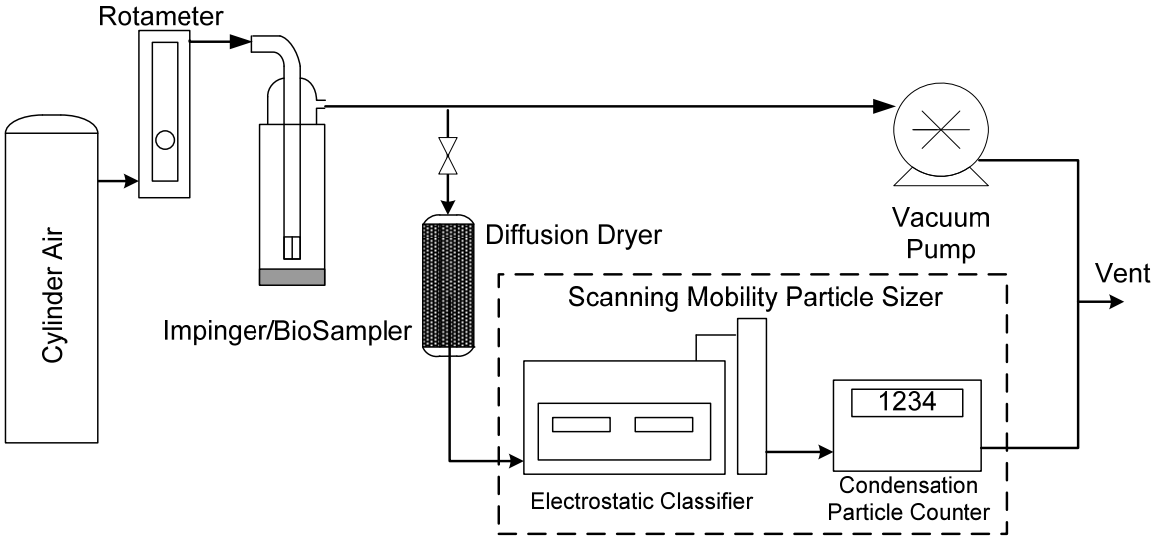

Figure 1 Schematic of setup for reaerosolization experiments

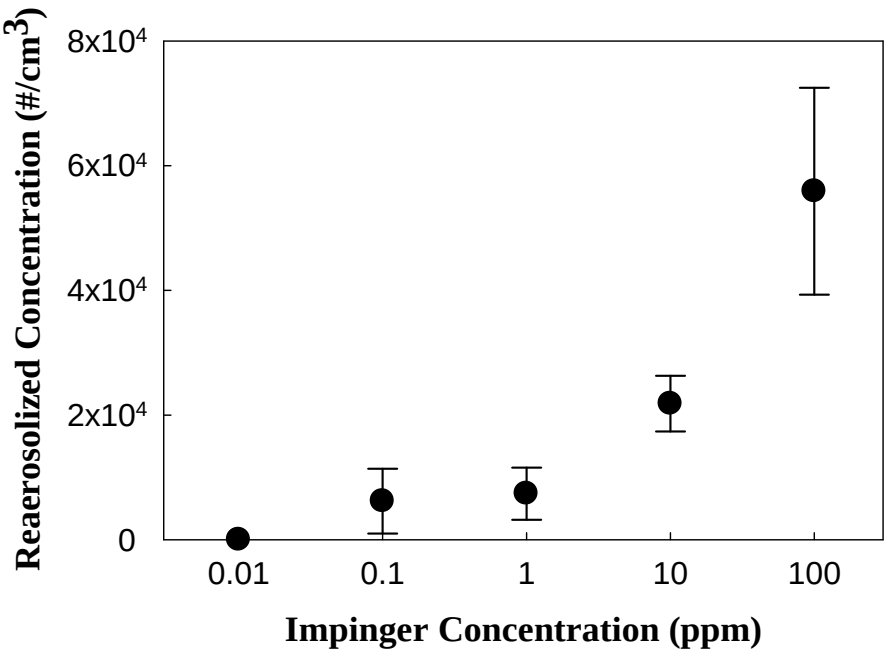

Figure 2 Reaerosolization of PSL particles as a function of impinger concentration for PSL particles at 12.5 Lpm. The error bar represents one standard deviation.

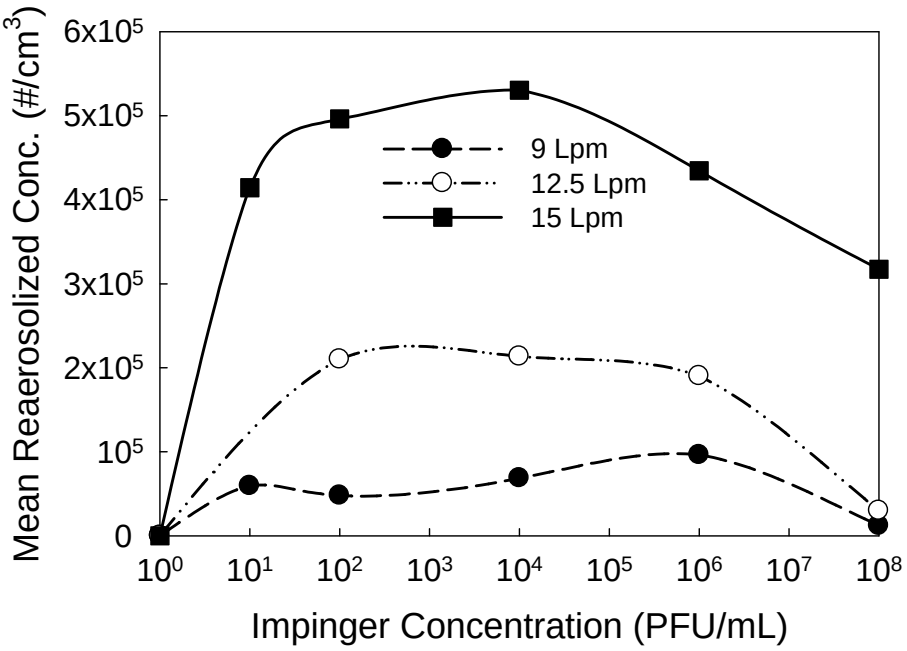

Figure 3 Reaerosolization as a function of flow and impinger concentration for MS2 viral particles

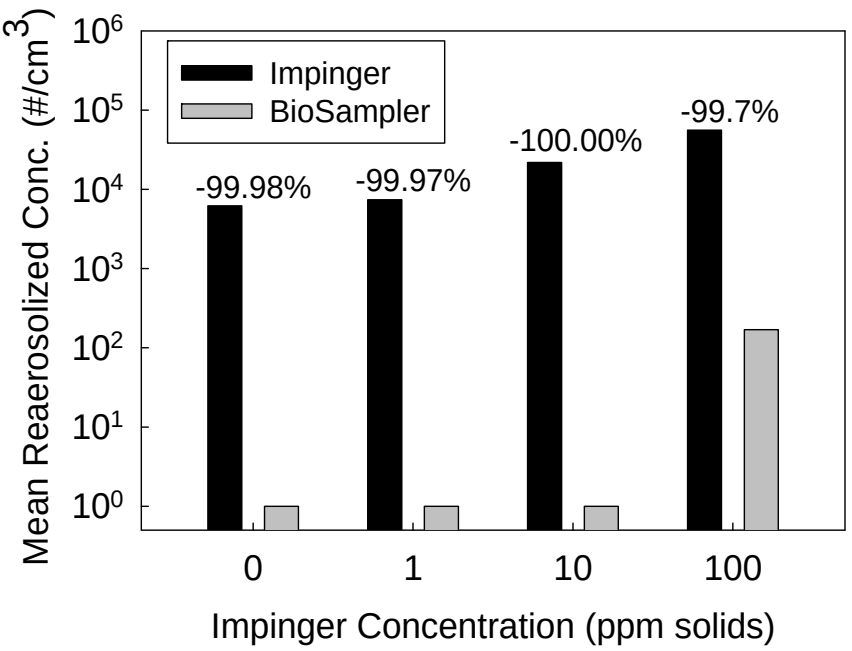

Figure 4 Comparison of the reaerosolization of 30-nm PSL particles at 12.5 Lpm from the impinger and BioSampler

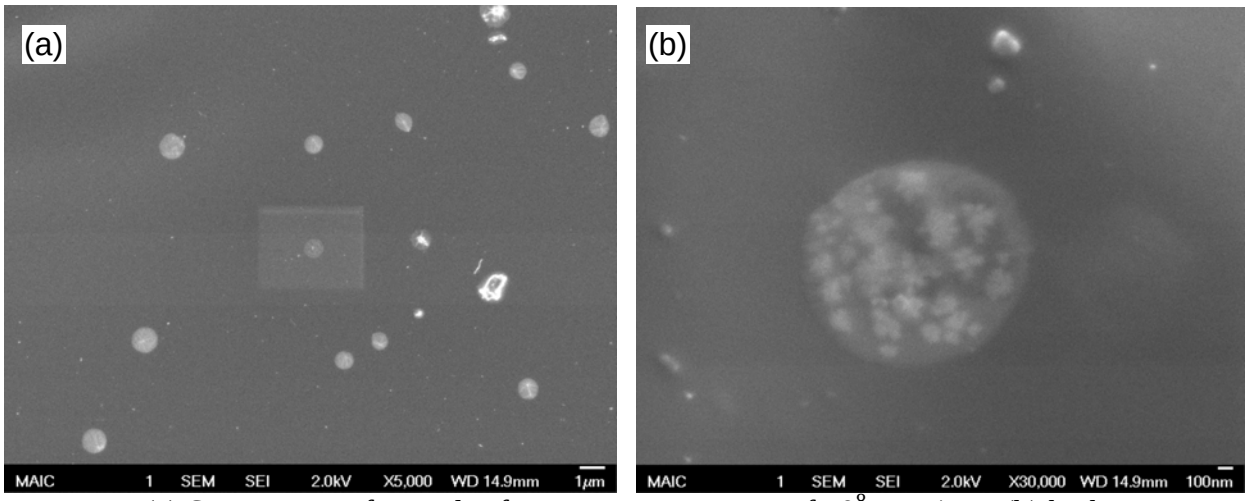

Figure 5 (a) SEM image of particles from virus suspension of 10^8 PFU/mL; (b) higher magnification of the particle at the center of 5(a)